On the Origin of Leukemic Species

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Recent studies (Shlush et al., 2014; Corces-Zimmerman et al., 2014) have demonstrated that leukemias develop from hematopoietic stem cells that acquire preleukemic mutations, allowing clonal expansion and subsequent acquisition of mutations leading to cancer. Preleukemic cells survive chemotherapy and serve as reservoirs for disease, generating new clones and leading to relapse.

Acute myeloid leukemia (AML) is a malignancy in which hematopoietic stem and progenitor cells (HSPCs) give rise to clonal populations of primitive cancer cells with impaired differentiation, known as "blasts." These clones vary in dominance over time, some with distinct, and others with overlapping, mutational profiles. The application of next-generation sequencing to define the serial acquisition of genetic mutations has allowed a preliminary view of the evolution of primary AML by The Cancer Genome Atlas (TCGA) consortium (Cancer Genome Atlas Research Network, 2013) and of relapsed disease (Ding et al., 2012). Mutational analysis of AML patient cohorts has also begun to define the prognostic value of these mutations (Patel et al., 2012). However, an in-depth understanding of the evolutionary origins of leukemic clones is still lacking.

Two recent studies by the Dick (Shlush et al., 2014) and Majeti (Corces-Zimmerman et al., 2014) groups now demonstrate that HSPCs acquire preleukemic mutations. These preleukemic stem cells contribute to normal blood development, harbor a selective growth advantage compared to their nonmutated normal stem/progenitor counterparts, and can subsequently gain additional mutations that confer full malignant potential (Figure 1). The fundamental shift in thinking provided by these collective studies is the demonstration that nonmalignant precursors give rise to a collection of preleukemic cells that persist after treatment and generate multiple waves of malignant clones over time. Some of these new clones are related to the original malignant clone, whereas others resemble the preleukemic HSPCs but contain a unique set of downstream mutations and are independent from the original dominant malignant clone. These preleukemic HSPCs are classified as nonmalignant based on their ability to generate cells in nonmalignant lineages (e.g., B and T cells).

Shlush et al. performed deep sequencing on 103 candidate genes frequently mutated in AMLs (Shlush et al., 2014). They used normal T lymphocytes as a surrogate for the occurrence of mutations in HSPCs and compared mutational profiles to those obtained from leukemic blasts in the same blood sample. Using this methodology, they demonstrated that somatic *DNMT3A* mutations occur in an ancestral cell that generates both T cells and the dominant leukemic clone. *DNMT3A* mutations were shown to occur in specific HSPC populations including, but not limited to, HSPCs from a relapsed patient, megakaryocyte-erythroid progenitors (MEPs) and CD33+ myeloid cells from a patient in remission, and MEPs from a patient at diagnosis. Several patient blast samples had mutations in *NPM1* not found in their normal T cells. Strikingly, *NPM1* mutations were only observed with *DNMT3A* mutations, further suggesting that *DNMT3A* mutations are early events in leukemogenesis.

The authors then demonstrated that preleukemic HSPCs survive after chemotherapy. By comparing allelic frequencies of $DNMT3A^{mut}$ at times of diagnosis, remission, and relapse, they found increased prevalence of the mutated allele at remission and relapse, suggesting that these early, preleukemic cells harboring $DNMT3A^{mut}$ could be a reservoir for recurrent disease. Furthermore, using xenograft repopulation assays, Shlush et al. established that $DNMT3A^{mut}$-bearing HSPCs engraft and generate long-term multilineage reconstitution, and they demonstrated that these cells possess a competitive repopulation advantage over nonmutated HSPCs, similar to previous observations showing that murine *Dnmt3a*-deficient HSPCs demonstrate a growth advantage over wild-type cells (Challen et al., 2012).

Previous work from the Majeti group, analyzing a small cohort of AML patients, suggested that preleukemic mutations are present in HSPCs (Jan et al., 2012). Expanding on this earlier work, Corces-Zimmerman et al. (2014) now identify preleukemic mutations in FACS-purified HSPCs from a larger cohort of patients manifesting a broader spectrum of AML. Similar to the Dick group, they found that "preleukemic" HSPCs can generate long-term myeloid and lymphoid engraftment in mice. The authors define mutations in *IDH2*, *DNMT3A*, *ASXL1,* and *IKZF1* in the HSPC population and in leukemic cells, and in certain cases, in a fraction of sorted B and T cells at diagnosis. This indicates a clear contribution of the preleukemic HSPCs to overall hematopoiesis. The authors observe distinct patterns of mutation acquisition, with about half of the recurring mutations being classified as early, mostly in genes involved in DNA methylation, chromatin modification, and chromatin topology, and the other half of mutations classified as late, mostly within genes encoding proteins important in cell signaling and proliferation. Similar to the findings of the Dick group, the authors find that *DNMT3A* mutations were present in preleukemic HSPCs in 75% of patients, and additionally, that 80% of patients also harbored preleukemic *IDH1/2* mutations. These mutations are found in the heterozygous state, implying that they can have dominant effects over the normal allele (Kim et al., 2013).

Importantly, Corces-Zimmerman et al. found that preleukemic HSPCs are not eradicated by chemotherapy. Using bone marrow samples from patients in remission, as well as from patients with relapsed disease, the authors find that preleukemic HSPCs survive chemotherapy, persist during remission, and generate new waves of clones leading to relapse. They also found new mutations acquired during relapse that were not present at diagnosis or during remission, indicating that in some cases, a different HSPC clone may lead to the origin of relapsed disease. Majeti and colleagues make the important point that there are multiple pathways to relapsed AML (Corces-Zimmerman et al., 2014): cells that are refractory to initial therapy; clonal evolution from the original clone; growth of new subclones present at diagnosis; and new clones arising from a preleukemic HSPC.

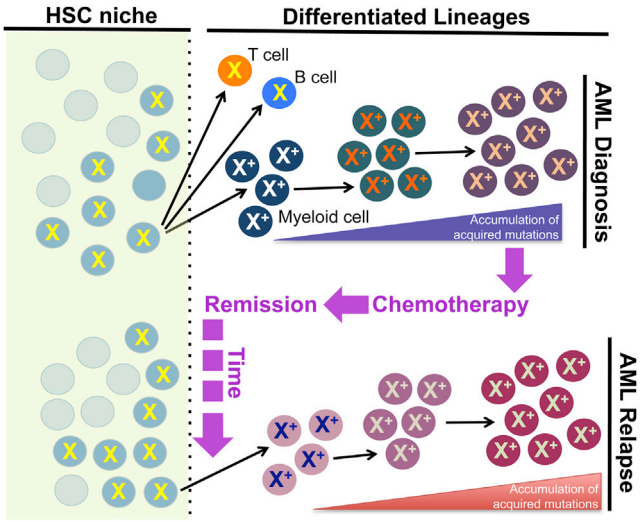

Figure 1. On the Origin of Leukemic Species

A model for the clonal origin and evolution of AML emerges from the work by Shlush et al. (2014) and Corces-Zimmerman et al. (2014), which describes sequential rounds of clonal expansion from a preleukemic stem cell pool. Hematopoietic stem cells (HSPCs; blue circles) gain mutations in epigenetic modifiers (designated by "X") to become preleukemic HSPCs (darker blue circles), which reside in a unique bone marrow niche (shown in yellow highlighting) and enjoy a growth advantage over normal HSPCs. Preleukemic stem cells have the capacity to differentiate into multiple cell types, including myeloid and lymphoid derivatives. Overt leukemia develops when preleukemic HSPCs accumulate oncogenic mutations (designated "X+") over time. Chemotherapy induces clinical remissions by killing the leukemia cells, but the expanded pool of preleukemic HSPCs remains. With time, a new clonal expansion may emerge, leading to clinical relapse. A major challenge for modern approaches to achieving long-term remissions is the eradication of the preleukemic stem cell pool.

These studies raise additional questions. For instance, will similar evolutionary patterns be identified for AMLs driven by recurring translocations or mutations other than *DNMT3A* or *IDH2*? What other early mutations drive the development of preleukemic HSPCs? Given that normal HSPCs acquire mutations with age (Welch et al., 2012), how often do these mutations generate HSPCs? *TET2*, another gene in the epigenetic pathway, for example, can be mutated in elderly individuals with clonal hematopoiesis but without overt malignancy (Busque et al., 2012). Do mutations that generate a preleukemic HSPC population always involve a gene that encodes a chromatin-remodeling protein? How does AML arise from preleukemic HSCs that show no genetic mutations? What additional pathways lead to disease relapse?

Reflecting on Darwin's writing, "I have called this principle, by which each slight variation, if useful, is preserved, by the term of Natural Selection...," it is remarkable to think that this statement is still relevant to our work today, more than 150 years after Darwin's words were published in 1859. One can almost imagine that Darwin wrote in response to the findings of these two outstanding papers.

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